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Detection of Herpes Simplex Virus Type-Specific Antibodies by an Enzyme-Linked Immunosorbent Assay Based on Glycoprotein G

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In order to develop a simple and quantitative method to detect herpes simplex virus (HSV) type-specific antibodies, the usefulness of an enzyme-linked immunosorbent assay (ELISA) using HSV glycoprotein G (gG) captured on a plate by monoclonal antibodies as antigen was studied. The gG1- and gG2-specific IgG antibody activities were measured by the ELISA for 54 sera which had been collected from culture-proven genital herpes patients and pre-characterized by an immunodot assay using purified gG antigens. Thirty control sera without antibodies against the HSV whole antigens were also included. In comparison with the immunodot assay as standard, the sensitivities of the ELISA were 88.9% (32/36) for HSV-1 antibody and 89.2% (33/37) for HSV-2 antibody and the specificities were both 100%. Sera taken within a few months after primary infection tended to give false negative results. The HSV type-specific ELISA based on easy-to-prepare gG antigens might be useful to help improve the serological assessment of HSV infections. ***J. Med. Virol. 53:319–323, 1997.***

KEY WORDS: HSV type-specific antibody; glycoprotein G; ELISA; immunodot; monoclonal antibody

INTRODUCTION

Herpes simplex virus (HSV) infections are caused by two distinct types of viruses, HSV-1 and HSV-2. HSV-1 is often acquired during childhood and is the predominant cause of oral infection, whereas HSV-2 infections are usually acquired later during adolescence and adulthood as sexually transmitted genital infections. Following initial infection, the virus assumes a lifelong latent state. Periodically, the latent virus reactivates resulting in viral shedding with or without clinical symptoms [Nahmias et al., 1989].

Recently, genital HSV infections became a matter of concern as a public health problem. This recurrent disease has generated a large pool of potentially infectious individuals, while putting each of the sufferers under great stress physically or psychologically. Genital HSV infections during pregnancy often become the source of fatal neonatal infections through vertical transmission [Nahmias and Roizman, 1973]. Genital ulcers caused by HSV infections have been shown to be a risk factor of acquiring and transmitting human immunodeficiency virus [Hook et al., 1992]

Whereas antigen detection remains the method of choice for the diagnosis of genital HSV infection [Peterson et al., 1983], serologic tests serve as an indispensable adjunct for both clinical and epidemiological studies. Genital HSV infections show a wide variety of clinical manifestations from recognizable symptomatic disease to subclinical, asymptomatic infections. Of the subjects with HSV-2 antibodies, only about 20% report a history of clinical disease; more than 80% of the HSV-2 infections may be inapparent and require serologic determination [Koutsky et al., 1990].

However, detection of HSV-2 antibody, particularly in the sera of individuals infected with HSV-2 over prior HSV-1 infection has been difficult. The serologic assessment of the type-specific immune status of HSV infections has been hampered by the extensive type-cross reactivity of HSV-specific antibodies [Honess et al., 1974; Ashley et al., 1993]. Due to the large number of viral proteins, most of which are conserved between the two virus types, the Western blot pattern is extremely complex, limiting widespread use [Ashley et

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al., 1985; Bernstein et al., 1985]. Recently, the antibody response to glycoprotein G has been demonstrated to be apparently entirely type-specific due primarily to the presence of a 526 amino-acid insertion in gG2 relative to gG1 [McGeoch et al., 1987]. An immunodot assay based on purified gG has proved to be a rapid and accurate type-specific serologic method [Lee et al., 1985; Lee et al., 1986]. However, the assay is not quantitative and thus it is not suitable for determining serial changes in antibody responses of various class- or subclass-specific immunoglobulins present at low concentrations in serum while controlling for nonspecific background reactions.

To establish an easy and quantitative method to detect HSV type-specific antibodies, we developed an enzyme-linked immunosorbent assay (ELISA) based on gG1 and gG2 captured on a plate from HSV-infected cell lysates by monoclonal antibodies against them.

MATERIALS AND METHODS

Sera were collected from 54 female genital herpes patients attending the department of Obstetrics and Gynecology, Tokyo University Branch Hospital during 1972 and 1985. Diagnosis was based on clinical symptoms and viral culture [Kawana et al., 1976]. Isolated viruses were identified by fluorescein isothiocyanate-labeled monoclonal antibodies against HSV-1 and HSV-2 (MicroTrak, Syva, CA). HSV type-specific antibodies of the sera had been pre-determined by the gG-specific immunodot assays [Lee et al., 1985; Lee et al., 1986], and classified into 3 groups; 17 sera from which HSV-1 was isolated with only gG1 antibodies (panel A), 18 sera from which HSV-2 was isolated with only gG2 antibodies (panel B), and 19 sera from which HSV-2 was isolated with both antibodies to gG1 and gG2 (panel C). Thirty sera from blood donors, confirmed to be seronegative by ELISA using HSV whole antigens [Hashido et al., 1989] were also examined. The clinical types of genital herpes [Nahmias et al., 1970; Corey, 1985] of the patients from whom serum was collected were as follows; panel A consisted of 10 cases of primary, 3 cases of recurrent and 4 cases of nonprimary first episode type HSV-1 genital herpes, panel B consisted of 9 primary and 9 recurrent cases of HSV-2 genital herpes, and panel C consisted of 11 recurrent and 8 nonprimary first episode cases of HSV-2 genital herpes. The primary type was diagnosed if HSV neutralizing antibody titer in the acute-phase serum was <8. If the titer was $\geqq 8$, without significant increase in the convalescent-phase serum, the patient was considered to have a recurrent or nonprimary first episode infection. With a previous history of genital herpes, the patient was diagnosed as a recurrent type. If there was no previous history, the patient was diagnosed as having a nonprimary first episode infection.

The antigens were made as reported previously [Hashido et al., 1989]. Briefly, HEp-2 cells infected with HSV-1 (syn^+17) or HSV-2 (UW268) at m.o.i 1.0 were lysed with lysis buffer (150mM NaCl, 1% NP-40, 50 mM Tris, pH8.0) after 24 hrs of infection and the centrifuge-supernatant was collected. Mock-infected cells prepared in the same way were used as a control antigen.

The gG-capture ELISA was carried out as follows. One hundred microliters of monoclonal antibodies (MoAbs) against HSV glycoprotein gG1 and gG2 (Clone #1107 and #1106, available commercially from Goodwin Institute for Cancer Research, Plantation, FL) diluted 1:2000 in 0.1 M bicarbonate buffer (pH 9.6) containing 30 μg/ml bovine serum albumin were hydrophobically adsorbed on a 96-well plate (Nunc, Maxisorp, Denmark) 4 wells each. After 2 hrs at 37°C, the plate was washed with phosphate-buffered saline (PBS) and blocked with PBS containing 5% skim milk for 1 hr at 37°C. HSV-1, HSV-2 and control antigens diluted to the protein concentration of 5 μg/ml in the diluting buffer (PBS with 0.05% Tween 20 and 1% fetal calf serum) were each added to 2 wells which had been coated with the homologous monoclonal antibody, and incubated overnight at 4°C. After washing and blocking, the serum specimen diluted 1:100 in the diluting buffer was added to 8 wells each and incubated for 4 hrs at room temperature (RT). Biotin-labeled monoclonal anti-human IgG $F(ab')_2$ (Zymed, San Francisco, CA) diluted at 1:1,000, and then streptavidin labeled with horseradish peroxidase (Zymed, San Francisco, CA) diluted at 1:4000, were added successively to the specimans under test and incubated for 1 hr at RT. After incubation with ortho-phenylenediamine and H_2O_2 in 0.1 M citrate-phosphate buffer, the reaction was stopped with 4N H_2SO_4, and optical density (OD) at 492 nm was measured. The gG1-specific antibody activity was calculated from [OD of wells with HSV-1 antigen captured by anti-gG1 MoAb]–[OD of wells with control antigen on anti-gG1 MoAb]. The gG2-specific antibody activity was calculated similarly by [OD of wells with HSV-2 antigen captured by anti-gG2 MoAb]–[OD of wells with control antigen on anti-gG2 MoAb]. Cut-off values were tentatively determined as the mean + 3SD of gG1-specific antibody activity of panel B sera for HSV-1 antibody, and the mean + 3SD of gG2-specific antibody activity of panel A sera for HSV-2 antibody.

For multiple comparison of the values of antibody activity against the gG proteins among 3 serum panel groups, Scheffe's *F* posthoc procedure was performed with the use of the statistical software StatView V.4.0.

RESULTS

The IgG antibody activities against gG1 and gG2 antigens of each serum panel are shown in Figure 1. The mean ± standard deviation (SD) of gG1 antibody activity was 0.624 ± 0.460 for panel A and 1.120 ± 0.366 for panel C, but 0.040 ± 0.055 for panel B. On the contrary, that of gG2 antibody activity was 0.661 ± 0.468 for panel B sera and 0.816 ± 0.406 for panel C, although for panel A it was 0.018 ± 0.040. Thus, in the gG1-capture ELISA, panels A and C showed significantly higher activity than panel B ($P < 0.0001$). In the gG2-capture ELISA, panels B and C showed significantly higher

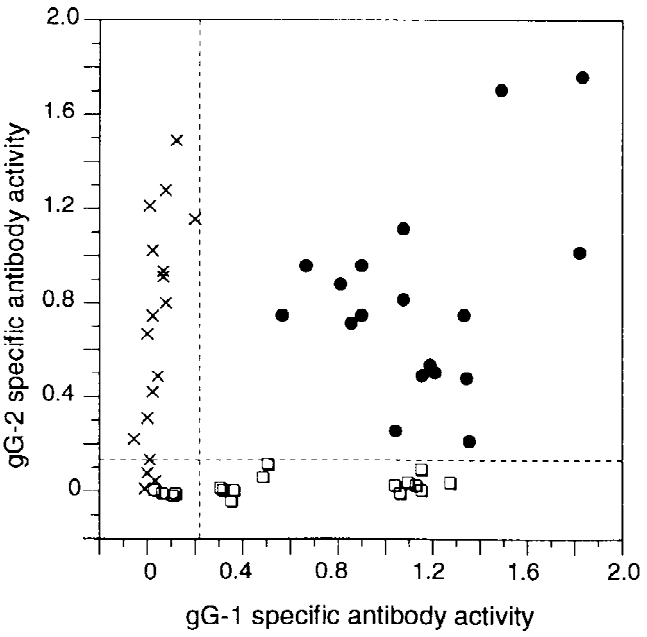

Fig. 1. HSV-specific IgG antibody levels against gG1 and gG2 antigens. □, panel A sera (with gG1 antibodies and without gG2 antibodies by immunodot); × panel B sera (without gG1 antibodies and with gG2 antibodies by immunodot); ●, panel C sera (both gG1 and gG2 antibodies present by immunodot); - - - - -, the cut-off values.

activity than panel A ($P < 0.0001$). Between the antibody activities against gG1 and gG2 antigens no tendency of correlation was observed. Antibody activities of the sera without HSV-specific antibodies were between −0.024 and 0.017 (0.002 ± 0.011) to gG1 antigen and between −0.016 and 0.016 (0.002 ± 0.008) to gG2 antigen (data not shown).

The sensitivities and specificities of the gG-capture ELISA were determined based on the tentative cut-off values; 0.206 for gG1 ELISA (the mean + 3 SD of antibody activity of panel B sera), and 0.134 for gG2 ELISA (the mean + 3 SD of antibody activity of panel A sera). By comparing the results with the gG-specific immunodot assay as a standard, the sensitivity was 88.9% (32/36) for HSV-1 antibody and 89.2% (33/37) for HSV-2 antibody (Table I). The specificities were both 100% (17/17, 18/18, respectively).

The gG1- and gG-2-specific antibody activities in the primary, recurrent, and nonprimary first episode types of genital herpes are shown in Figure 2. For 18 samples collected from patients with primary infection (HSV-1-isolated patients, 10; HSV-2-isolated patients, 8), the antibody responses were plotted in relation to time after onset of the infection. A tendency toward an increase in the gG-specific antibody activity was observed. However, the sera collected within a few months after primary infection tended to be low, and also the nonprimary cases gave a wide range of gG-specific antibody activity. According to the tentative cut-off values, 3 sera from the patients with HSV-1 primary infection and 3 sera from HSV-2 primary infection, and one serum sample from each of HSV-1 or HSV-2 recurrent infection gave false-negative results.

DISCUSSION

Due to the lack of a simple and quantitative method to subtype HSV antibodies using type-specific antigens, serodiagnosis of HSV-2 infection has not been carried out widely. Although several methods have been developed since the mid 1980s using recombinant or MoAb-purified gG proteins [Lee et al., 1985; Lee et al., 1986; Sullender et al., 1988; Spiezia et al., 1990; Parkes et al., 1991; Sancez-Martinez et al., 1991; Ho et al., 1992; Kakkanas et al., 1995], these assays have not been available to most general clinical laboratories, mainly due to the very limited supply of reliable gG-1- and gG-2-specific monoclonal antibodies, difficulties in reconstructing or reproducing the systems using recombinant protein expression, and lack of commercial availability due to the lack of potential profitability for the industry.

Most available commercial diagnostic tests based on the comparison between their relative reactivities against whole HSV-1 or HSV-2 antigen give inaccurate results [Ashley et al., 1991; Field et al., 1993], caused by extensive cross-antigenicity between proteins of both HSV types. A recently developed strip immunoblot assay using recombinant gG1 and gG2 antigens was shown to be an accurate type-specific assay [Alexander et al., 1996]. However, the assay is not quantitative and limited to the detection of IgG for research use only, not for diagnostic procedures (manufacturer's package insert, Chiron Corp., Emeryville, CA). Cost is also an important factor in selecting a method for routine use.

A simple ELISA was developed based on the gG1 and gG2 antigens captured on a plate by commercial monoclonal antibodies, which is quantitative, potentially suitable for detection of all classes and subclasses of immunoglobulin, and feasible for use in most clinical laboratories. This method is simpler than expression systems by bacteria or insect cells which are labor- and time-consuming procedures and pre-purification procedures by affinity chromatography the efficiency of which depends greatly on the ability of MoAb to both bind and release the antigen and require a large quantity of MoAb with high concentration. The commercial MoAb clones #1107 and #1106 we used are identical to the clones H1379 and H1206 developed by Pereira et al. [1982] and well characterized for use for seroepidemiology by Lee et al. [1985, 1986] (Dr. Dennis Emma, Goodwin Institute for Cancer Research, personal communication).

We found in a preliminary study that careful selection of the best cell/virus combination for recovery of the gG proteins from infected cells is necessary. Production of the gG1 and gG2 proteins is variable depending on the combination of cell and virus strain used, which would influence the intensity of antigen-antibody reaction, and therefore affect the assay performance. By comparison of reactivity of the gG-1 and gG-2 proteins among the combinations of 2 cell lines (HEp-2 and HEL R-66) and 3 strains each of HSV-1

TABLE I. Comparison of the Results by HSV gG1- and gG2-Capture ELISA and the Immunodot Assay for Panel A, B and C Sera

Panel sera	Immunodot antibodies to gG1	Immunodot antibodies to gG2	ELISA antibodies to gG1	+	−	+	−	Total
			gG2	−	+	+	−	
A	+	−		13	0	0	4	17
B	−	+		0	14	0	4	18
C	+	+		0	0	19	0	19
Total				13	14	19	8	54

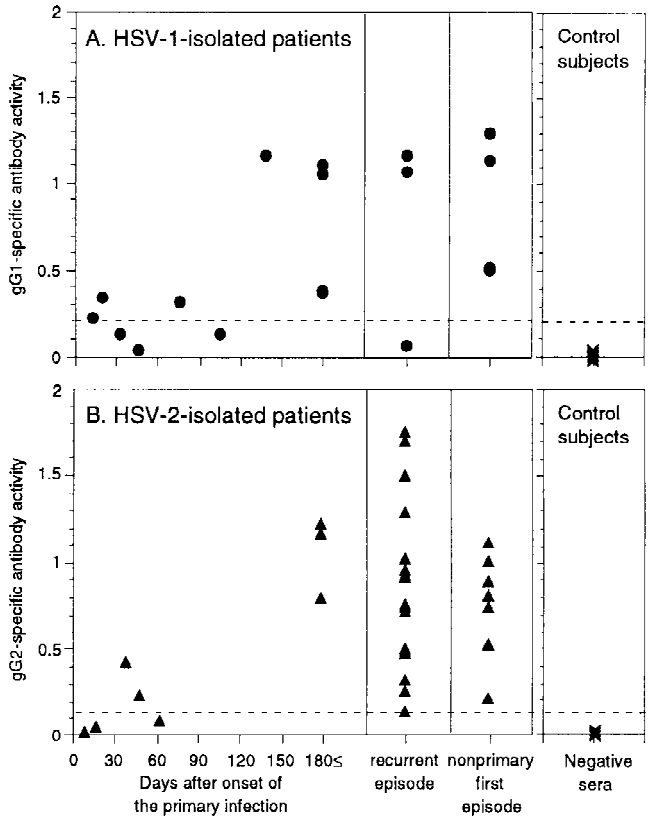

Fig. 2. Relationship between the levels of HSV-specific IgG antibodies against gG1 or gG2 antigens with clinical types of genital herpes and time after onset of the infection. ●; serum with gG1 antibodies. ▲; serum with gG2 antibodies. - - - - -, the cut-off values.

(syn + 17, HF, TVK-171) and HSV-2 (HG52, UW268, THH-54) checked immunologically by Western blot and ELISA, it was decided to use the gG-1 protein produced by syn+17 and gG-2 protein by UW268 in HEp-2 cell for the present study.

Four of 36 gG1 immunodot-positive sera and 4 of 37 gG2 immunodot-positive sera fell below the tentative cut-off levels. As shown in Fig. 2, 6 of these sera found to be false-negative had been collected within a few month of primary infection; at the longest, the 106th day for HSV-1 and the 62th day for HSV-2 infection. The remaining 2 sera were both collected from recurrent cases. Anti-HSV antibodies were measured in all serum specimens by an indirect ELISA using the whole HSV antigen, and found that these false-negative sera contained HSV-specific antibodies at very low levels (data not shown). Although the sample number studied was too small to draw any conclusions, with the gG-capture ELISA the detection of such low levels of gG1- and gG2-specific antibodies tended to be difficult, and the sensitivity should be improved in future.

Also, the reactivity of our gG-capture ELISA relative to the time after infection, which implies the usefulness of this assay for serodiagnosis of HSV infection, should be studied with a larger number of sera, which should be collected independently of the assay results obtained by the immunodot assay to avoid sample-collection bias. However, caution is necessary in evaluating an assay based on the gG for serodiagnosis of HSV infection, since the detection rate of anti-gG antibodies within 40 days after infection was reported to be 71% for HSV-1 infection and 62% for HSV-2 infection by Western blot [Ashley et al., 1988], which suggests the possibility of a generally late increase in anti-gG antibodies in HSV infection. At present, we recommend the use of an assay against the whole HSV antigen simultaneously with assays against the gG proteins.

The "original antigenic sin" phenomenon [Inouye et al., 1984; Ashley et al., 1994] is known for the assays using HSV type-common antigens, that is, in persons with prior HSV-1 infection, the secondary infection with HSV-2 causes a rapid and strong antibody response to HSV-1 with a possibility of suppressing or delaying the production of HSV-2 antibodies in those patients. However, this study showed that relatively high levels of antibodies to HSV-2 were observed in most of the patients with HSV-1 antibodies from which HSV-2 was isolated. It was also observed that the gG-1 antibody responses were not boosted significantly in HSV-2-isolated patients, which seems reasonable since any proteins produced by HSV-2 do not share cross-reactive antigenicity with the gG-1 protein. The clinical differences between HSV-2-infected patients with and without the presence of prior gG1 antibody are concern for future analysis.

In conclusion, although the improvement of the assay performance and its adaptation for the detection of IgM or other subclass antibodies are necessary for this gG-capture ELISA to be practically used, it showed a potential to help general clinical laboratories in the serological assessment of HSV infections, having the

advantage of utilizing easy-to-prepare antigens, the test itself being free of labor-intensive procedures.

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